

## CellCycle Plugin

June 26, 2025

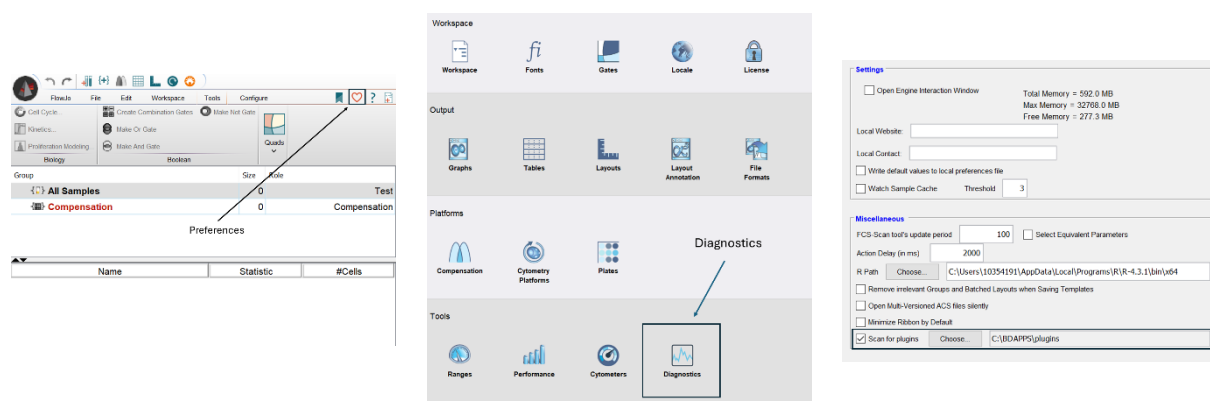
Cell\_cycle\_v1.0.2.jar

### Introduction

The Cell Cycle plugin automatically identifies the phase of the cell cycle events are in. The plugin is able to perform two dimensional (2D) or three dimensional (3D) cell cycle analysis using a DNA stain, like DAPI, a proliferation marker (like BrdU or EdU), and/or a mitotic marker (like PH3). The algorithm utilizes Hierarchical Density-based Scanning in Applications with Noise (HDBSCAN) to first identify high-density populations within the feature space and then classify them based on relative positioning to one another. This plugin provides critical enhancements to BD FlowJo's built-in Cell Cycle Analysis tool, which currently only performs in one dimension (DNA staining) and does not provide gate membership.

### Download and Installation

1. Place the plugin .jar file in your Plugins folder.
2. Navigate to Preferences > Diagnostics and select the “Scan for Plugins” checkbox to point FlowJo to your Plugins folder (Figure 1).
3. Click OK.
4. Restart FlowJo.



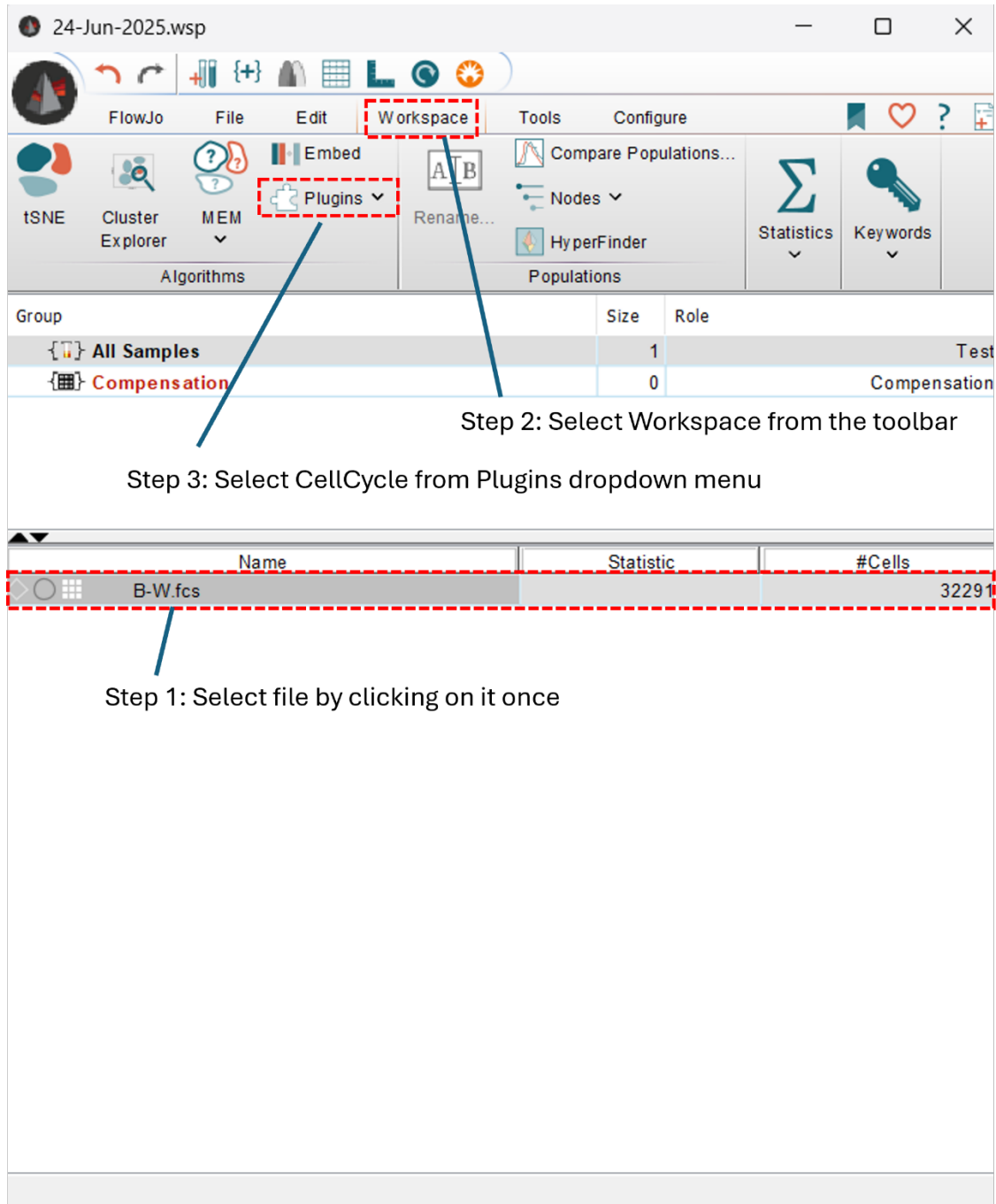
**Figure 1** – FlowJo Preferences navigation for Plugins folder.

### Requirements

- Plugin runs on Windows and Mac with ARM processors.
- Samples must be prepared using at least 2 markers for correct classification:
  - 2D Classification: DNA marker (like DAPI) + proliferation marker (like BrdU or Edu).
  - 3D Classification: 2D Classification markers + mitotic marker (like PH3).

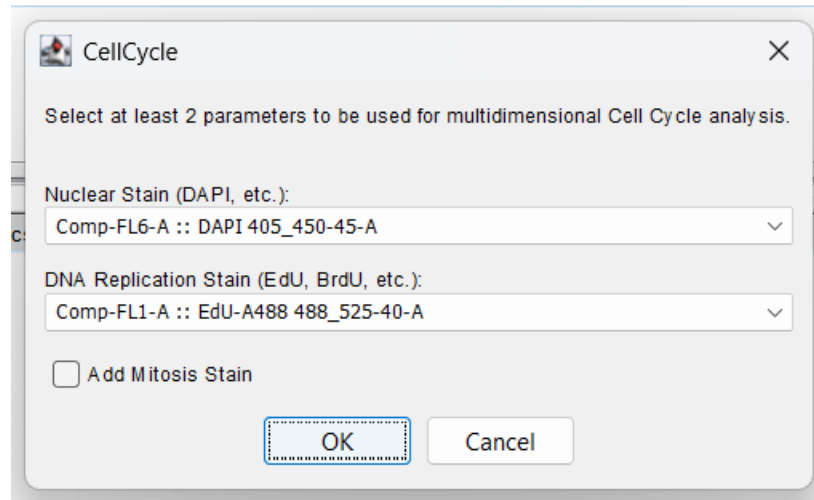
### Usage

1. Begin by saving your workspace.
2. Once saved, click once on the sample in the workspace and then navigate to Workspace > Plugins > CellCycle to start the plugin (Figure 2).



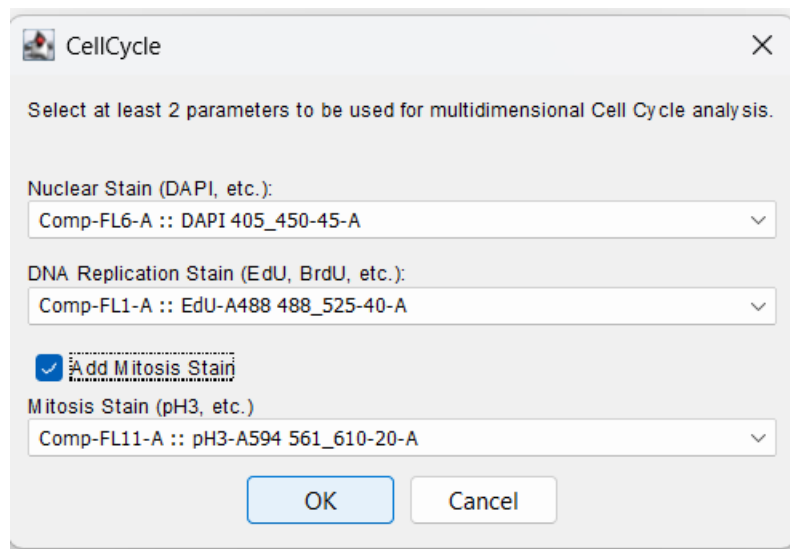
**Figure 2** – Initiate the plugin by first selecting the sample, navigating to Workspace, then Plugins, and then CellCycle.

3. The CellCycle dialog box will appear with two combo boxes to select the DNA and proliferation markers, respectively. If parameters are labeled, the plugin will display the appropriate compensated parameters by default (Figure 3).



**Figure 3** – CellCycle dialog box displaying the DNA and proliferation markers by default.

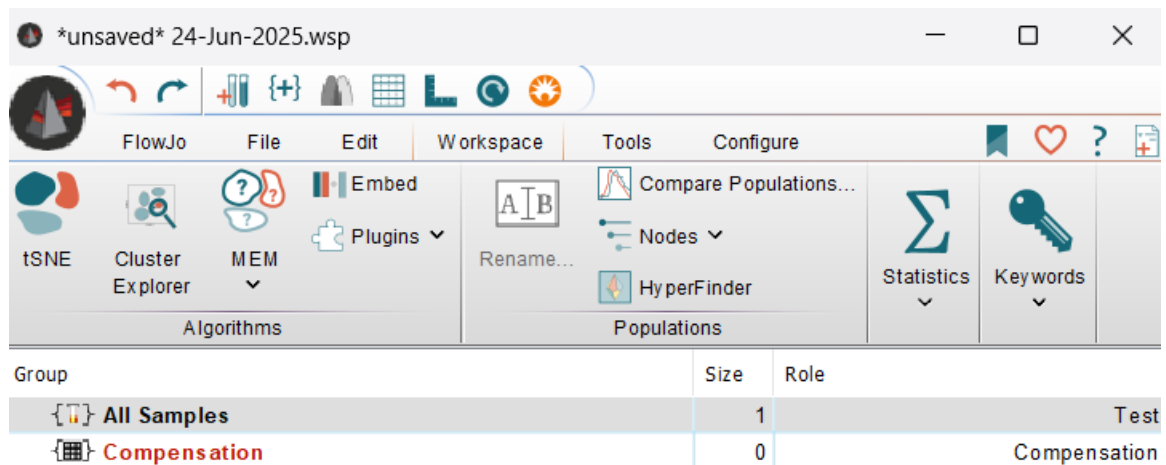
4. To add a mitotic marker, simply select the checkbox. Similar to the first two combo boxes, the appropriate parameter should be selected by default (Figure 4).



**Figure 4** – Select the “Add Mitosis Stain” checkbox to add a mitotic marker.

5. Once all parameters are selected, press OK to continue.
6. After the plugin is finished running, subpopulations will appear under the CellCycle Results\_####, where #### represents a randomly-generated alphanumeric unique identifier for this particular run of the plugin (Figure 5).
  - a. Output Subpopulations:
    - i. G1: the senescent phase of the cell cycle.

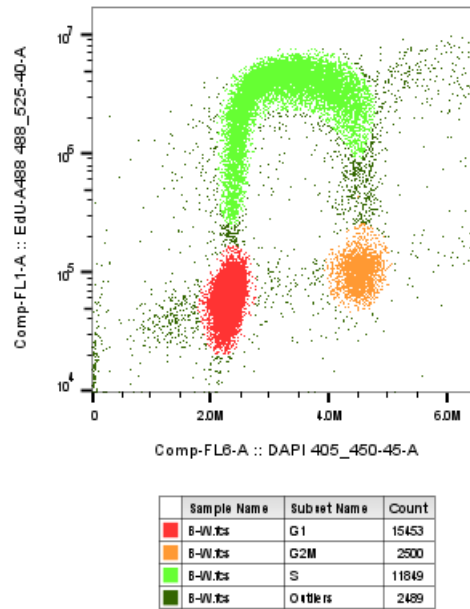
- ii. G2M (2D analysis only): the phase where cells have duplicated DNA and are in or entering mitosis.
- iii. G2 (3D analysis only): Cells have replicated DNA, but have not yet entered mitosis.
- iv. M (3D analysis only): Cells undergoing active mitosis.
- v. S: proliferation phase, spanning the entire range from G1 to G2.
- vi. S-early: A population of cells in S phase, but has not fully replicated DNA yet.
- vii. S-late: A population of cells in S phase with mostly replicated DNA.
- viii. Non-S: A population that is not currently in S phase and is somewhere between G1 and G2M phases.
- ix. Other: A population of cells that does not directly correspond to traditional cell cycle phases. May require further investigation or relabeling.
- x. Outliers: Cells that were not in a sufficiently dense enough region to be clustered into a subpopulation.



Name	Statistic	#Cells
B-W.fcs		32291
CellCycle Population_V9EI		
CellCycle_V9EI		CellCycle completed
CellCycle Results_V9EI	100	32291
G1	47.9	15453
G2M	7.74	2500
Outliers	7.71	2489
S	36.7	11849

**Figure 5** – Subpopulations appear under the CellCycle Results\_#### node.

7. Populations can be viewed in the FlowJo Layout Editor to confirm classification (Figure 6).



**Figure 6** – The FlowJo Layout Editor can be used to show how the subpopulations overlay onto the larger populations.

## Resources

- [HDBSCAN Implementation from Scikit-Learn](#)

## Contact Us

- Please contact us at [flowjo@bd.com](mailto:flowjo@bd.com) for any questions or concerns regarding this plugin.